REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks. Applicants sincerely thank the Examiner for holding a telephonic interview with Applicants' representative. The Examiner's kind suggestions have been incorporated herein.

I. CLAIM STATUS AND AMENDMENTS

Claims 1-5, 7, 11, 13-17 and 19 were pending in this application when last examined.

Claims 1-5 and 7 were examined on the merits and stand rejected.

Claims 11, 13-17 and 19 were withdrawn as non-elected subject matter. Applicants reserve the right to file a Continuation or Divisional Application on any withdrawn subject matter.

Claim 1 is amended to clarify the claimed invention. It is further noted that figure 1 clearly shows a double-stranded DNA primer consisting of a first strand having a primer sequence and a second strand as well as ligation of the 3' end of the first strand cDNA to the 5' end of the first strand of the double-stranded DNA primer.

No new matter has been added.

II. ANTICIPATION AND OBVIOUSNESS REJECTIONS

On pages 2-5 of the Office Action, claims 1-5 and 7 were rejected under 35 U.S.C. § 102(b) as anticipated by Kato et al. (WO 94/08001).

On pages 6-9, claims 1-5 and 7 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Chenchik et al. (US 5,962,271) in view of Brennan et al. (Methods in Enzymology, Vol. 100, pages 38-52, 1983).

Applicants respectfully traverse these rejections as applied to the amended claims.

Initially, it is noted that the claimed invention has been clarified to require that the 3' end of the first strand cDNA is ligated to the 5' end of the first strand of the double-stranded DNA primer using T4 RNA ligase. Referring to figure 1 of the specification, it is therefore apparent that T4 RNA ligase ligates together a cDNA/mRNA heteroduplex to a double-stranded DNA.

On the other hand, in Kato et al., the cap structure of intact mRNA is removed by using decapping enzyme, and a "DNA-RNA chimeric oligonucleotide" is ligated to the 5'-end of the mRNA by using **T4 RNA ligase** to form a "DNA-RNA chimeric oligonucleotide-ligated mRNA" (see the bottom of Fig. 1 and the top of Fig. 2), as the following structure.

5'- [DNA-RNA] + [mRNA] -3'

Thus, T4 RNA ligase performs an RNA-RNA ligation. Please note that T4 ligase can be also used for DNA-RNA ligation. In this regard, Kato et al. describes "Since T4 RNA ligase catalyzes the ligation between RNA strands more efficiently than between RNA and DNA strands, the use of a DNA-RNA chimeric oligonucleotide is preferred" (column 3, lines 61-64). In the case using DNA oligonucleotide, the structure is as follows.

5'- [DNA] + [mRNA] -3'

In next step of Kato et al., double-stranded DNA primer is annealed with the 5'-end of the DNA-RNA chimeric oligonucleotide-ligated mRNA, and synthesized the first cDNA strand from the primer, as the following structure.

5'- [DNA-RNA] + [mRNA] + [DNA]-3'
3'- [cDNA] +[DNA]-5'

or

5'- [DNA] + [mRNA] + [DNA]-3'
3'- [cDNA] + [DNA]-5'

And then, both ends are ligated to be a circular vector, where DNA/DNA is ligated to DNA/DNA. Kato et al. do not explicitly describe that the ligation is made by T4 DNA ligase. However, it was common knowledge in the art that the T4 DNA ligase is only one to ligate DNA/DNA and DNA/DNA. This is same in Chenchick et al. where DNA/DNA and DNA/DNA are ligated with T4 DNA ligase. Further, Kool teaches that **single-stranded** DNA or RNA can be ligated with T4 RNA ligase, but does not refer to use of T4 RNA ligase for ligation of double stranded DNAs.

Thus, Kato et al. fails to disclose use of T4 RNA ligase to ligate a strand of a RNA/cDNA heteroduplex to a double-stranded DNA. Instead, Kato et al. discloses either using T4 RNA

ligase to perform RNA to RNA ligation or using T4 DNA ligase to ligate double-stranded DNA to double-stranded DNA. Further, Chenchik et al. teaches using T4 DNA ligase to ligate double-stranded DNA to double-stranded DNA. Finally, Kool teaches that single-stranded DNA or RNA can be ligated with T4 RNA ligase.

Thus, Applicants respectfully submit that these rejections are untenable as none of the cited art teaches or suggests use of T4 RNA ligase to ligate a DNA/RNA heteroduplex to double-stranded DNA as required by amended claim 1.

You may have one question why did the present inventors think up the use of T4 RNA ligase in the present invention. The present inventors firstly made an attempt to modify the method of Kato et al. This is because that in the steps of Fig. 1, the single-stranded mRNA was degraded easily in the process of each treatment.

Then, the inventors tried the following method of:

(1) annealing double-stranded DNA primer with the single-stranded mRNA;

(2) preparing an mRNA/cDNA heteroduplex by synthesizing a first cDNA;

(3) replacing cap structure of mRNA in the heteroduplex with DNA-RNA chimeric oligonucleotide by using T4 ***RNA ligase*** to form;

5'- [DNA-RNA] + [mRNA] + [DNA]-5'

3'- [cDNA] + [DNA]-5' and

(4) circulizing the both ends (DNA/DNA) by using ***T4 DNA ligase*** to form a circular vector.

In this process, mRNA is subjected with some treatments in a form of mRNA/cDNA heteroduplex, and therefore is more stable.

But in practice, a circulation was happened in the step (3) with ***T4 RNA ligase***. There may be two possibilities:

(i) T4 RNA ligase can ligate DNA-RNA chimeric oligonUcleotide to mRNA, and then ligate DNA/DNA to DNA/DNA; or

(ii) T4 RNA ligase can directly ligate both ends of:

5'- [mRNA] + [DNA]-3'

3'- [cDNA] + [DNA]-5'

In the circular vector obtained in step (3), there was not DNA-RNA chimeric oligonucleotide. The present inventors found the possibility (ii), i.e., T4 RNA ligase can ligate RNA/DNA to DNA/DNA, and have completed the present invention. This discovery of the activity of T4 RNA ligase to ligate a cDNA/mRNA heteroduplex to double-stranded DNA was surprising and unexpected. It was not taught or suggested by the cited art.

Thus, for the above noted reasons, these rejections are untenable and should be withdrawn.

CONCLUSION

In view of the foregoing amendments and remarks, it is respectfully submitted that the present application is in condition for allowance and early notice to that effect is hereby requested.

If the Examiner has any comments or proposals for expediting prosecution, please contact the undersigned attorney at the telephone number below.

Respectfully submitted,

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May 28, 2010